



CME review article

CRTH2 antagonists in the treatment of allergic responses involving T_H2 cells, basophils, and eosinophils

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ARTICLE INFO

Article history:

Received for publication October 31, 2011.

Received in revised form April 2, 2012.

Accepted for publication April 2, 2012.

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Release Date: December 1, 2012

Expiration Date: November 30, 2014

Estimated Time to Complete: 60 minutes

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- Discuss the importance of CRTH2 in the cells of the immune system and its role in allergic inflammation
- Describe the role of PGD2 and its receptors as well as its role after it is released from the mast cell

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Disclosure of Relevant Financial Relationships:

R.G. Townley, S. Agrawal, and G.D. Marshall have nothing to disclose. Reviewers and Education/Editorial staff have no relevant financial relationships to disclose. No unapproved/investigative use of a product/device are discussed.

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Introduction

Asthma is a chronic inflammatory disease of the airways that causes airway hyperresponsiveness, bronchoconstriction, and mucous secretion and is associated with inflammatory cells, such as basophils, mast cells, eosinophils, macrophages, and T_H cells.

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Funding Sources: Financial support is from the Allergy Division, Department of Medicine, Creighton University.

Asthma also causes the release of inflammatory mediators, such as growth factors, lipid mediators, chemokines, and chemical mediators.¹ Lipid mediators, such as leukotrienes, prostaglandins, and platelet-activating factor, are important modulators in asthma and allergic rhinitis.²

Topical corticosteroids and nonsedating antihistamines effectively reduce inflammation and disease severity and have good safety profiles compared with systemic steroids. However, both allergic rhinitis and severe asthma are not sufficiently controlled by current therapy. Therefore, there is a clear need for effective treatment of allergic diseases with acceptable side effect profiles that can be administered safely by an oral route.³

Most allergic diseases are characterized by elevated IgE levels. A particular antigen first encounters antigen-processing cells, such as macrophages and dendritic cells. CD4 T_H2 cells produce cytokines that play an important role in allergic responses. T_H2 cells produce the cytokines interleukin (IL) 4, IL-5, IL-6, IL-9, and IL-13. IL-4 promotes proliferation and differentiation of T_H2 cells.¹ IL-4 and IL-13 can cause isotype switching in B cells, leading to the production of IgE.⁴ IL-5 stimulates eosinophil release from the bone marrow, causes direct activation and priming, and prolongs eosinophil survival. IL-13 causes mucous production and airway hyperresponsiveness.⁵ IL-13 also causes airway hyperresponsiveness, which is associated with a decreased response to β -adrenergic bronchodilators and corticosteroids.^{6,7}

CRTH2 (chemoattractant receptor homologous molecule expressed on T_H2 cells) is a receptor of prostaglandin D₂ (PGD₂), a member of the prostanoid receptor family. The number of CRTH2 cells in the skin is significantly increased in patients with atopic dermatitis. Barnes et al⁸ provided the first clinical evidence of the CRTH2 antagonist OC000459 as a novel oral treatment for asthma. Recently, Schirasaki et al⁹ reported accumulation of CRTH2-positive leukocytes in human allergic nasal mucosa. These studies in patients with atopic dermatitis, bronchial asthma, and allergic rhinitis provide intriguing evidence of the role of CRTH2 in these allergic diseases.

Role of PGD₂ in Asthma

Prostaglandins are the lipid-derived mediators generated by metabolism of arachidonic acid by prostaglandin synthase enzymes and cyclooxygenase. The prostanoid receptor subfamily consists of 9 members, which are classified according to the prostanoid ligand that each binds with greatest affinity: TP, DP, CRTH2, EP1 to 4, FP, and IP (Table 1).¹⁰ TP is the thromboxane A₂ receptor, DP is the prostanoid receptor, EP1 to 4 are the prostaglandin E₂ receptors, FP is the prostaglandin F_{2 α} receptor, and IP is the prostaglandin I₂ prostacyclin receptor.

PGD₂ is a major prostanoid produced by the mast cells and its metabolites, which initiate type 1 allergic responses.¹¹ PGD₂ is also produced in the brain, where it might be involved in sleep regulation and pain perception or other activities of the central nervous system.¹² In peripheral tissues the richest source of PGD₂ is mast cells, produced by the IgE-activated mast cells at levels of approximately 50 ng per 1 $\times$ 10⁶ cells. Other cells, such as T_H2 cells, dendritic cells, skin Langerhans cells, and keratinocytes, also produce PGD₂, but their levels are lower than those produced by the mast cells.¹³

The presence of allergen causes release of PGD₂ in large amounts during asthma attacks. Bronchial allergen challenge in asthmatic patients leads to rapid production of PGD₂ that can be detected within minutes in the bronchoalveolar lavage (BAL) fluid and is nearly 150 times higher than the preallergen levels.¹⁴ PGD₂ is released into the airways after antigen challenge, and PGD₂ inhalation challenge elicits airway eosinophil infiltration and bronchoconstriction. Local antigen challenge in the nasal mucosa of patients with allergic rhinitis stimulates the production of PGD₂ in

the nasal secretions and in the skin of patients with atopic dermatitis. PGD₂ and its metabolites have been proposed as selective markers of mast cell activation *in vivo*.^{15,16}

The hematopoietic PGD synthase is expressed mainly in the mast cells, dendritic cells, macrophages, and T_H2 cells. PGD₂ induces its proinflammatory effects, such as vasodilation, leading to erythema and potentiation of edema, induction of T_H2 cytokine synthesis, recruitment of eosinophils and T_H2 cells, and bronchoconstriction in asthma.¹⁷ PGD₂ is released during early and late asthmatic responses after allergen exposure in patients with asthma¹⁸ and has been demonstrated to be a potent chemoattractant for eosinophils *in vivo* and *in vitro*.¹⁹ Thus, PGD₂ produced by mast cells may provide a link between early-phase and late-phase allergic responses.

PGD₂ and Receptors

PGD₂ exerts its biological effects via 3 G protein-coupled receptors: DP1/DP, TP, and CRTH2/DP2.¹³ The DP receptor is articulated not only on hematopoietic cells, basophils, eosinophils, and dendritic cells but also on other cell types,²⁰ such as nasal epithelial goblet cells and smooth muscle cells. DP1 is expressed by platelets, and activation leads to inhibition of platelet aggregation. DP1 is expressed by leukocyte populations, such as dendritic cells, where it controls various functions, including production of cytokines, such as IL-12. Activation by selective DP1 agonist BW245C promotes relaxation of both airway and vascular smooth muscle, which leads to bronchodilation²¹ and vasodilation. As stated by Matsuoka et al,²¹ PGD₂ and DP agonist BW245C each induced tracheal relaxation of wild-type mice, whereas no such effect is apparent in muscle derived from DP knockout animals. DP knockout mice showed only marginal infiltration of eosinophils and failed to develop airway hyperreactivity. These authors concluded that PGD₂ functions as a mast cell-derived mediator to trigger asthmatic responses.

Mice deficient in DP1 exhibit decreased T_H2-mediated airway inflammation and airway hypersensitivity in the ovalbumin-induced model of asthma.²¹ DP1 is expressed on airway epithelial cells and mediates PGD₂-driven mucous secretion in patients with rhinitis and asthma.

CRTH2/DP2

CRTH2 or DP2 was originally identified as orphan receptor known as GPR44 (G protein-coupled receptor 44). CRTH2 is the most recently identified receptor for PGD₂, and it mediates its effect by promoting the activation of T_H2 lymphocytes, eosinophils, and basophils²² and mediates chemotactic responses of these cells to PGD₂.¹⁹ Other chemotactic receptors, such as the leukotriene B₄ receptors BLT1 and BLT2, formyl peptide receptors, and the complement C5a receptor, closely resemble CRTH2.

Activation of CRTH2 results in signaling events (Fig 1). CRTH2 is coupled to G α i, and its activation leads to reduction in cyclic adenosine monophosphate (cAMP) and elevation of intracellular calcium. Downstream activation of CRTH2 results in phosphoinositide 3 kinase-dependent phosphorylation of AKT, nuclear translocation of nuclear factor of activated T cells, and activation of glycogen synthase kinase (GSK) 3 β .²⁴ Akt, also known as protein kinase B, is a serine/threonine protein kinase that plays a key role in multiple cellular processes, such as glucose metabolism, cell proliferation, apoptosis, transcription, and cell migration.

Phosphatidylinositol 3 kinase-dependent phosphorylation of GSK-3 β leads to inactivation of GSK-3 β , which in turn leads to reduction in the phosphorylation of nuclear factor of activated T cells. An interesting aspect is the expression pattern of CRTH2. CRTH2 is expressed on human T_H2 cells, whereas mice express CRTH2 on both T_H1 and T_H2 cells, suggesting that CRTH2 function

Table 1
Comparison of the pharmacologic and biological properties of prostanoids and their receptors^a

Class	Receptor	Endogenous ligands	Signaling mechanism	Synthetic agonist	Synthetic antagonist	Location	Proinflammatory, anti-inflammatory, and biological effects
Thromboxane A2	TP	Thromboxane A2, 9 α 11 β PGF ₂	Gq, Gs(α), Gi(β), ↑IP3/DAG/Ca ²⁺ , ↑cAMP, G _h (α), G ₁₂ , ↓cAMP	U46619, I-BOP, STA2	Ramatroban, SQ29548, S-145, ICI192605, GR32191	Vascular smooth muscle, bronchial smooth muscle, platelets	Bronchiole smooth muscle hyperplasia and proliferation, bronchoconstriction and vasoconstriction, increased T-cell proliferation, inhibition of dendritic cell–T-cell interaction and platelet aggregation
PGD ₂	DP1	PGD ₂	Gs, ↑cAMP, ↑Ca ²⁺	BW245C,L-644,698, ZK110841	BWA868C, S-5751, MK-0524	Vascular smooth muscle, bronchial smooth muscle, dendritic cells, CNS, platelets	Vasodilatation, inhibition of eosinophil apoptosis, bronchodilatation, decreased cytokine production by dendritic cells, inhibits platelet aggregation
	CRTH2	PGD ₂ , DK-PGD ₂ , 15R-PGD ₂ , 15-deoxy- $\Delta^{12,14}$ PGD ₂ , Δ^{12} PGD ₂ , PGJ ₂ , Δ^{12} PGJ ₂ , 15-deoxy- $\Delta^{12,14}$ PGJ ₂ , 11-dehydro-thromboxane B ₂ , 9 α 11 β PGF ₂	Gi, ↓cAMP, ↑Ca ²⁺ , PLC, PI3K, MAPK	Indomethacin, L-888,607	Ramatroban and analogues (TM30642, TM30643, TM30089), indole-3 acetic acids, aryl acetic acids derivatives, 4-amino-tetrahydroquinolone derivatives (K117 and K376), and MK-7246	T ^H 2 lymphocytes, eosinophils, basophils	Activation and chemotaxis of T _H 2 cells, eosinophils, and basophils
PGE ₂	EP1, EP2, EP3, EP4		Gs, ↑cAMP, Gi, Gq, ↑IP3/DAG	ONO-KI-004, ONO-AEI-259, MB28767, ONO-AE-248, ONO-AEI-329, misoprostol	SC51322, SC51089, ONO-8713, L-826266, AH23848B, ONO-AE3-208	Lungs, bronchiole, CNS, macrophages, dendritic cells, T and B cells, kidneys	LPS-stimulated oxidative damage, IgE class switching by B cells, inhibition of T cell proliferation
PGI ₂	IP		Gs, Gq, Gi, ↑IP3/DAG	Iloprost, Cicaprost, Carbacyclin		Kidney, lung, liver, platelets, heart aorta	Increase in microvascular permeability, stimulation of T-cell IL-10 production, vasodilation
PGF ₂ α	FP		Gq, ↑IP3/DAG	Fluprostenol, latanoprost		Kidneys, uterus	Facilitation of inflammatory pain transmission and inhibition of lymphocyte–endothelial cell interaction

Abbreviations: cAMP, cyclic adenosine monophosphate; CNS, central nervous system; DAG, diacylglycerol; DP, prostanoid receptor; DK-PGD₂, 13,14-dihydro-15 keto-PGD₂; EP1–4, PGE₂ receptors; FP, PGF₂ α receptor; IL, interleukin; IP, PGI₂ prostacyclin receptor; LPS, lipopolysaccharide; PI3K, phosphoinositide 3 kinase; IP3, inositol triphosphate; MAPK, MAP kinase; PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; PGF₂ α , prostaglandin F₂ α ; PGI₂, prostaglandin I₂; PLC, phospholipase C; TP, thromboxane A2 receptor.

Symbols: Upward arrow, increased; downward arrow, decreased.

^aData were compiled and synthesized from Pettipher,³ Pettipher et al,¹³ and Ulven and Kostenis.²⁷

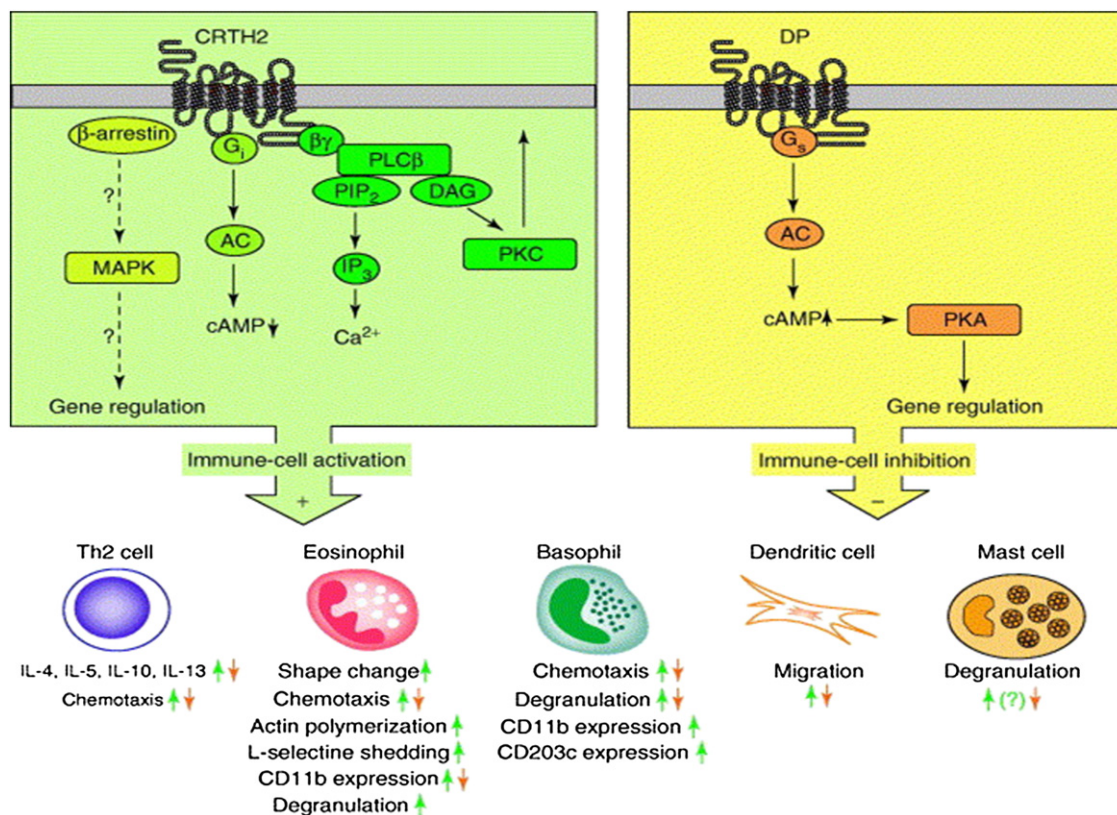


Fig. 1. The control of immune cell function in an antagonist manner by CRTH2 (chemoattractant receptor homologous molecule expressed on T_H2 cells) and DP (prostanoid receptor). CRTH2 inhibits adenylyl cyclase (AC) through G_i proteins; this inhibition leads to a decrease in intracellular cyclic adenosine monophosphate (cAMP) levels. G_{12} subunit complexes released on G_i activation directly stimulate phospholipase C β (PLC β) isoforms, which generate diacylglycerol (DAG) and inositol triphosphate (IP $_3$) through breakdown of phosphatidylinositol-4,5-bisphosphate (PIP $_2$). IP $_3$ mobilizes Ca^{2+} from endoplasmic-reticulum stores. CRTH2 also signals through arrestin proteins in a G protein-independent manner, which is a newly discovered mode of signaling that partly underlies eosinophil shape change after prostaglandin D_2 (PGD $_2$) challenge. It is possible but not yet proven that arrestin recruitment might also lead to G protein-independent MAP kinase activation; this has been suggested for several other 7 transmembrane receptors. On the basis of the application of selective inhibitors, PLC β and various kinases (eg, p38 MAP kinase, PI3 kinase, and MEK kinase) have been implicated in CRTH2 signal propagation. Together, activation of various CRTH2 signaling pathways leads to immune cell activation. The responses of immune cells that are elicited by CRTH2 activation are listed below the individual cells, and the nature of the response is indicated by green arrows. The DP receptor stimulates AC through G_s proteins and increases intracellular cAMP levels. Importantly, increases of cAMP levels in immune cells are generally linked to inhibition of cell functions. The consequences on immune cells of DP activation are indicated by orange arrows. On a cellular level, DP and CRTH2 can be regarded as antagonistic receptors: CRTH2 mediates proinflammatory and prostimulatory effects, and DP limits CRTH2 activation on exposure to PGD $_2$. Data from Ulven and Kostenis.²⁷

might differ in human and rodents. CRTH2 receptor is activated by several PGD $_2$ metabolites, such as 13,14-dihydro-15 keto-PGD $_2$ (DK-PGD $_2$), 11-deoxy-11-methylene-15-keto PGD $_2$, (15R) methyl PGD $_2$, Δ^{12} PGD $_2$, PGJ $_2$, Δ^{12} PGJ $_2$, 9 α 11 β -PGF $_2$, 15-deoxy- $\Delta^{12,14}$ PGD $_2$, deoxy- $\Delta^{12,14}$ PGJ $_2$, and PGF $_2\alpha$ (Fig 2). All these metabolites retain activity on CRTH2 but are less active on DP1. Some of the metabolites formed independently of PGD $_2$ are 11-dehydrothromboxane B $_2$ and 9 α 11 β -PGF $_2$, which also have CRTH2 agonist activity.²⁵

Molecular Structure of CRTH2

Human CRTH2 contains 395 amino acid residues with a molecular mass of 43 kDa. It is a G protein-coupled receptor that has 7 transmembrane (TM) domains. Human CRTH2 has a long cytoplasmic tail and 2 N-glycosylation sites at the N⁺ extracellular domain. The long cytoplasmic tail has multiple sites for protein kinase C phosphorylation. On the basis of the molecular structure of CRTH2, a number of agonists and antagonists have been developed.^{26,27}

CRTH2 Agonist and Antagonist

Modifications of PGD $_2$ and screening of known and related prostanoids have allowed generating some basic ligand-binding

structure activity relationships. Nonsteroidal anti-inflammatory drugs have shown moderate binding affinity for CRTH2, such as sulindac sulfide and fenclofenac, but they were also active on DP1; however, indomethacin has selective binding for CRTH2 and is a potent CRTH2 agonist.²⁸ Zomepirac has been shown to have antagonistic activity in vitro. G protein-coupled receptors interact with an extraordinary diversity of ligands by means of their extracellular domains and/or the extracellular part of the TM segments.

Categorization of 7 TM receptors by a phylogenetically based method identified angiotensin type 1 and type 2 receptors to share similar binding properties to CRTH2. This led to recognition of TM27632 and TM3170 and Candesartan as CRTH2 ligands of which Candesartan was the most potent ligand for CRTH2.²⁹

The first synthetic, potent, and selective CRTH2 agonist identified by Gervais et al³⁰ was L-888,607, which exhibited high selectivity over all other prostanoids receptors (Table 1). L-888,607 has subnanomolar affinity for the human CRTH2 receptor and relative stability in vivo. It has agonist activity on recombinant and endogenously expressed CRTH2 receptor and thus can be an appropriate tool to explore the in vivo function of CRTH2.

Indole-3-acetic acid derivatives have been recognized as potent and selective antagonist of CRTH2. Within this structural class,

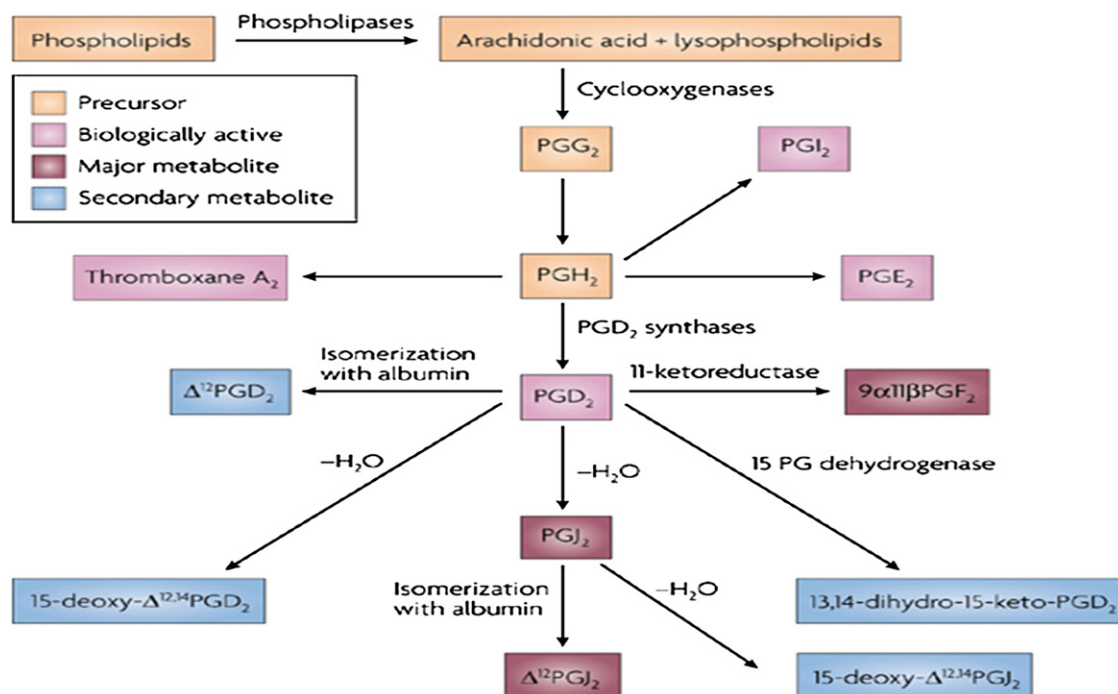


Fig. 2. Pathways of prostaglandin D₂ (PGD₂) metabolism. On cellular activation arachidonic acid is released from membrane phospholipids and converted to the cyclic endoperoxide prostaglandin G₂ (PGG₂) by the action of cyclooxygenase 1 (COX1) or COX2. The peroxidase activity of the same enzymes leads to the formation of prostaglandin H₂ (PGH₂), which can be converted to a number of biologically active prostaglandins by discrete synthase enzymes that show tissue-specific distribution and might demonstrate preferential coupling to either COX1 or COX2. PGD₂ production is dependent on the action of either the lipocalin PGD₂ synthase or the hematopoietic PGD₂ synthase. Hematopoietic PGD₂ synthase is present in mast cells, T_H2 cells, and other leukocytes, and it is thought to be responsible for the bulk of PGD₂ production during allergic responses. PGD₂ is rapidly metabolized (with a half-life of 1.5 minutes in blood), and the main products that have been detected in vivo are Δ¹²PGJ₂ and 9α11βPGF₂. The precise pathway of PGD₂ metabolism has not been fully elucidated, but it is possible that 13,14-dihydro-15-keto-PGD₂ (DK-PGD₂), Δ¹²PGD₂, and 15-deoxy-Δ^{12,14}PGJ₂ might be formed locally, which is of interest because these compounds, similar to Δ¹²PGJ₂, retain significant CRTH2 (chemoattractant receptor homologous molecule expressed on T_H2 cells) agonist activity. Data from Pettipher et al.¹³

Indole4 was the first identified CRTH2 antagonist with 40-fold functional selectivity for CRTH2 over DP1. Ramatroban is a nonselective, competitive CRTH2 antagonist with moderate potency that was originally identified as a TP antagonist. This observation led to the recognition of ramatroban analogues, which are potent CRTH2 antagonists without TP activity. Minor changes in the structure of ramatroban can lead to highly selective CRTH2 antagonist devoid of TP activity. Ramatroban is a synthetic antagonist of thromboxane A₂. Although ramatroban is marketed for treatment of allergic rhinitis in Japan, its activity has been reported to be increased in vitro by using the TM analogues of thromboxane, which have a decreased thromboxane activity as referred to in Table 1. We are not aware of clinical studies of the TM analogues of thromboxane.

Three potent CRTH2-binding compounds with high selectivity over the TP-binding site were recognized to have improved selectivity over ramatroban, and the functional activities of these 3 antagonists were found to be different. Competitive antagonism was demonstrated by ramatroban and TM30642, whereas TM30643 and TM30089 (a combination of both TM30642 and TM30643) demonstrated noncompetitive antagonism.^{3,13,23} Ramatroban is marketed in Japan with a trade name of Baynas for the treatment of perennial allergic rhinitis.

Another class of CRTH2 antagonist is Aryl acetic acids in which biaryl thioether 10 was first identified as a CRTH2 antagonist and has 40 times selectivity for CRTH2 over DP1. Nonacid CRTH2 antagonists that belong to the tetrahydroquinoline structural class have also been recognized. The 4-aminotetrahydroquinoline derivatives are the only known nonacid CRTH2 antagonists, as illustrated by K117 and K376³¹ (Table 1).

Gervais et al.³² recently demonstrated an inverse indole analog of TM30089 (ramatroban analog) characterized as MK-7246 as a novel synthetic CRTH2 antagonist. They showed that it displayed high selectivity over all other prostanoids receptors and enzymes and also that it demonstrated high affinity for human, mouse, rat, monkey, and dog CRTH2. MK-7246 also interacts with CRTH2 in a reversible manner and acts as a full antagonist on recombinant and endogenously expressed CRTH2. It has good oral bioavailability and metabolic stability in various animal species. MK-7246 produces ex vivo blockage of CRTH2 on eosinophils in sheep and monkeys. It significantly reduced antigen-induced late-phase bronchoconstriction and airway hyperresponsiveness in sheep. Thus, MK-7246 is a potent and selective tool that can be used to clarify the role of CRTH2 in inflammatory response both in vitro and in vivo.

Role of CRTH2 In Vitro

Cells of the immune system express both DP and CRTH2, which have opposing signaling pathways. DP activation leads to an increase in cAMP and inhibition of the immune system, whereas activation of CRTH2 leads to a decrease in cAMP, an increase in intracellular calcium, and activation of the immune system (Fig 1). Thus, these 2 PGD₂ receptors have opposite functions in inflammatory cells.

CRTH2 on Basophils

Basophils are important in asthma and allergic diseases. They are found in nasal mucosa and airways of allergic and asthmatic patients and rapidly infiltrate tissues after allergen aggravation (Fig 3).

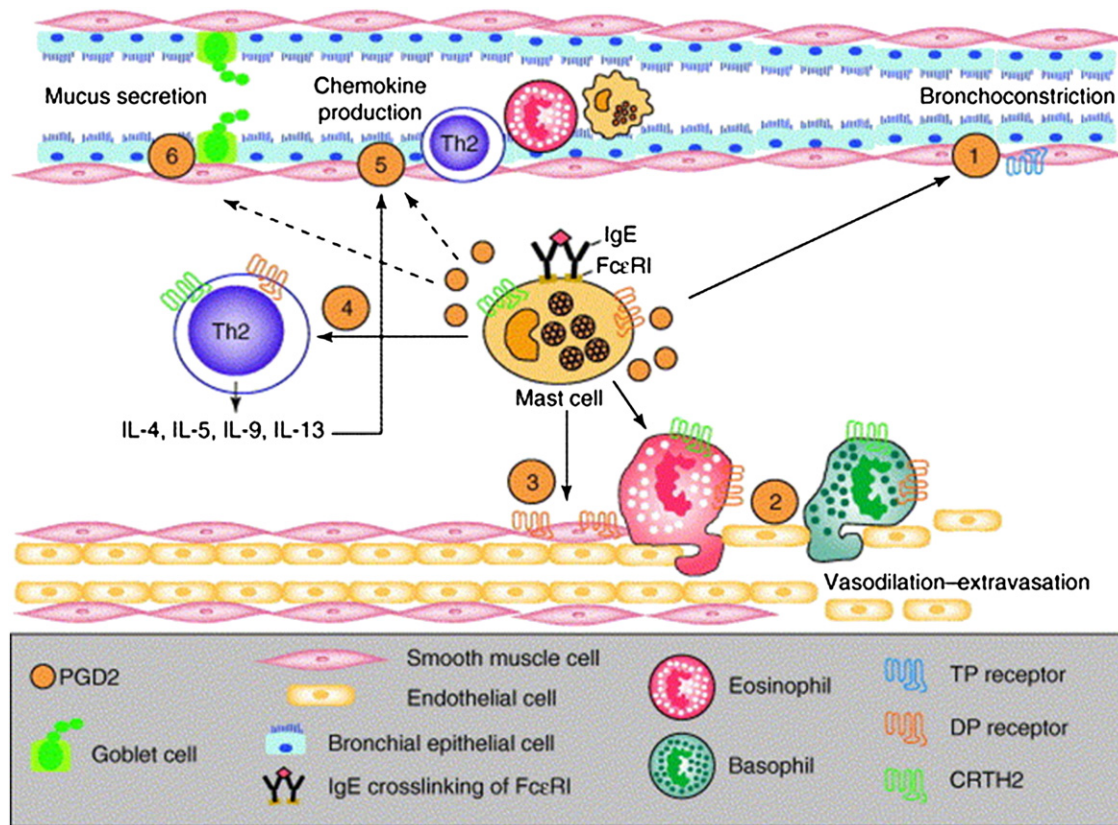


Fig. 3. Effects of prostaglandin D₂ (PGD₂) on immune cells and the pulmonary microenvironment. Mast cells and T_H2 cells that express both CRTH2 (chemoattractant receptor homologous molecule expressed on T_H2 cells) (green, stimulatory receptor in Fig 1) and DP (prostanoid receptor) (orange, inhibitory receptor in Fig 1) are central in allergic asthma. They can release PGD₂ on immunologic challenge (mast cells are activated after allergen exposure by its crosslinking to the high-affinity IgE receptor FcεRI), which exerts its asthma-promoting effects through various routes (1–6 in Fig 1; solid arrows indicate known molecular target for specific PGD₂ action; broken arrows indicate unknown molecular target for specific PGD₂ action). (1) PGD₂ acts as a bronchoconstrictor through TP (thromboxane A₂) receptors in bronchiole smooth muscle cells. (2) PGD₂ chemotactic activity recruits circulating eosinophils and basophils from the vascular bed to the site of inflammation in a CRTH2-dependent manner. However, transmigration of cells into the tissue requires vasodilation. (3) Because DP is expressed on airway smooth muscle cells, where the increase in intracellular cyclic adenosine monophosphate levels leads to relaxation, the vasodilation-extravasation component of inflammation is probably facilitated by DP. (4) Furthermore, PGD₂ has an important role in the early phase of CRTH2-dependent T_H2 cell recruitment and activation. Activated T_H2 cells produce various cytokines (such as interleukin 4 [IL-4], IL-5, IL-9, and IL-13) that, in turn, stimulate mast cells and eosinophils. (5) In addition, these cytokines initiate the next wave of T-cell recruitment via induction of T_H2 cell chemotactic chemokine expression, such as macrophage-derived chemokine (MDC) or thymus and activation-regulated chemokine by pulmonary epithelial cells. In particular, MDC expression that is regulated by PGD₂ is responsible for transepithelial migration of eosinophils, T_H2 cells, and macrophages. Data from Ulven and Kostenis.²⁷

Although mast cells are important in early-phase reaction, basophils are important in late-phase reaction. After allergen stimulation, they release various inflammatory mediators, such as histamine and leukotriene C₄. Basophils also cause the production of the T_H2 cytokines IL-4 and IL-13,³³ which have several important functions in initiating and maintaining allergic responses.²⁷ Basophils expressed CRTH2 and migrated toward PGD₂. Activation of CRTH2 enhanced basophil expression of adhesion molecule CD11b and histamine release.³⁴ CRTH2 signaling is responsible for mobilization of intracellular calcium and causes upregulation of adhesion molecule CD11b and basophil activation marker CD203c expression and augmentation of IgE-mediated basophil degranulation. On the contrary, activation of DP1 inhibits CD203c expression on basophil and IgE-mediated degranulation, and DP1 receptor antagonist BWA868C enhances PGD₂-elicited CD203c expression.

CRTH2 on Eosinophils

Eosinophils have an important role in late-phase reaction by releasing bronchoconstrictor mediators, such as leukotriene C₄ and chemoattractants, which causes influx of inflammatory cells into the tissue.³⁵ Chemoattractants comprise the ligands of the chemokine receptor CCR3, RANTES, eotaxin-1,2,3, and monocyte chemotactic proteins 3 and 4. Lipid mediators leukotriene B₄, platelet activation

factor, 5-oxo-6,8,11,14-eicosatetraenoic acid, and complement factor C5a are also potent chemoattractants for eosinophils.³⁴ Eosinophils produce and release immunoregulatory type 2 cytokines IL-4, IL-5, IL-10, and IL-13, proinflammatory cytokines, and growth factors. In chronically inflamed tissue, eosinophils also have a role in airway remodeling and angiogenesis.³⁴

CRTH2 and DP are both expressed by eosinophils, which interact with each other to regulate cell responses to PGD₂. Activation of CRTH2 leads to eosinophil shape change, chemotaxis, enhancement of chemotactic responsiveness to other chemoattractants, and degranulation,^{36–38} whereas activation of DP inhibits cell migration of Langerhans cells, dendritic cells, monocytes, and fibroblasts and leads to eosinophil survival. PGD₂ induces migration, adhesion molecule upregulation, calcium mobilization, and actin polymerization through CRTH2 on eosinophils. The selective antagonist BWA868C causes inhibition of DP and markedly enhanced activation of CRTH2, which causes an increase in CD11b expression when exposed to PGD₂.

Thus, there is a balance in the activation of immune cells by these 2 receptors; DP transmits inhibitory signals, whereas CRTH2 transmits proinflammatory stimuli. Immune cells express both CRTH2 and DP. CRTH2-mediated signals are predominant over DP-mediated signals when the cells are exposed to PGD₂. PGD₂ and its metabolites are competent in stimulating the release of mature

eosinophils from the bone marrow.^{38,39} PGD₂ and DK-PGD₂ are potent stimulators of eosinophil migration and shape change with cytoskeletal rearrangement, whereas BW245C did not induce eosinophil migration. Thus, these results suggest that the effects of DK-PGD₂ and PGD₂ on eosinophil migration are mediated through CRTH2 but not DP receptors, whereas DP agonist may regulate eosinophil activation.⁴⁰ Expression of CRTH2 is elevated in eosinophils from patients of allergic diseases, such as atopic dermatitis.⁴¹

CRTH2 on T_H2 Cells

CRTH2 has an important role in T_H2 lymphocyte recruitment and activation. As shown in Figure 3, PGD₂ has the ability to stimulate the production of the cytokines IL-4, IL-5, and IL-13 by human T_H2 cells in the absence of costimulation. Cytokine production in T_H2 cells is stimulated by PGD₂ and the CRTH2 selective agonist DK-PGD₂ indomethacin and inhibited by ramatroban. The DP selective agonist BW245C attenuates cytokine production, whereas the DP antagonist BWA868C cytokine production is not sensitive to inhibition.²⁷ CRTH2 stimulation in cultured T_H2 cells causes upregulation of CD40 ligand, which is a costimulatory molecule that is essential for T-cell–dendritic cell and T-cell–B-cell interaction that causes improved production of T_H2-type cytokines. CRTH2 also plays an important role in paracrine activation of T_H2 cells. Vinall et al⁴² showed that activation of human CRTH2⁺ CD4⁺ T_H2 cells with anti-CD3/anti-CD28 caused production of PGD₂ in a time-dependent manner and peaked at 8 hours. The production of PGD₂ was completely inhibited by diclofenac but was not affected by cotreatment with ramatroban. After 8 hours of activation the production of chemotactic activity was partially inhibited by diclofenac and completely inhibited by ramatroban, although the supernatants collected 24 hours after activation were less sensitive to inhibition by ramatroban. This finding suggests that blockage of CRTH2 by ramatroban causes inhibition of the chemotactic responses, which leads to inhibition of T_H2 cell recruitment at sites of allergic inflammation. Ramatroban antagonizes both the CRTH2 and TP receptors; the effect is due to the CRTH2 blockage because the selective TP antagonist SQ29548 did not inhibit migration of T_H2 cells.

Role of CRTH2 in Vivo

Mouse models of allergic asthma exhibit eosinophilia and mucous cell hyperplasia, which are the cardinal histopathologic signs of human asthmatic lungs. Studies indicate both anti-inflammatory and proinflammatory roles of DP and CRTH2 in vivo. Matsuoka et al²¹ found the mice that lack the functional DP cannot initiate an airway inflammatory response to an allergen. DP^{−/−} mice are protected from airway inflammation in response to allergen. Also during allergic inflammation in mice the DP receptor expression in the lungs is upregulated. In humans, single-nucleotide polymorphisms of DP are associated with lower risk of asthma because they reduce its transcriptional efficiency.⁴³ DP2 activation in vivo promotes eosinophilia and exacerbates disease in 2 models of T_H2-related inflammation, atopic dermatitis and allergic asthma, whereas DP1 activation results in opposite outcomes and counter-regulates T_H2 inflammation. This finding suggests that DP2 antagonist may be of therapeutic importance in diseases where eosinophilia has to be prevented.⁴⁴ These studies indicate the proinflammatory and anti-inflammatory role of DP in vivo. Intratracheal administration of PGD₂ in rats with systemic IL-5 injection on activation of CRTH2 induces eosinophil trafficking into the airways. CRTH2 also limits IL-5 production and eosinophil recruitment, which explains the anti-inflammatory effect of CRTH2.⁴⁵

Response in Allergic Rhinitis

Suzuki et al⁴⁶ investigated the prophylactic effect of the epinastine histamine₁ receptor antagonist ramatroban and the

thromboxane A2 receptor antagonist seratrodoast in a mouse model of allergic rhinitis. Sneezing and nasal rubbing are the 2 nasal allergic symptoms by which severity of allergic rhinitis is assessed. Histamine sensitivity and infiltration of eosinophils into the nasal mucosa were also determined. Epinastine and ramatroban significantly reduced nasal symptoms and the number of eosinophils in the nasal mucosa, whereas seratrodoast showed no effect. Histamine sensitivity was also reduced by epinastine and ramatroban, thus suggesting the role of epinastine and ramatroban in inducing prophylactic effect on allergic rhinitis.

Response in Allergic Skin Condition

Satoh et al⁴⁷ using CRTH2-deficient mice demonstrated that the PGD₂–CRTH2 system plays an important role in the IgE-mediated inflammatory response in the skin. Reduced skin responses were observed in the CRTH2-deficient mice. Hapten-specific IgE-induced ear swelling responses were less pronounced in mutant mice, yielding 35% to 55% responses in the healthy mice. Similar results were seen when mice were treated with ramatroban or HQL-79, a hemopoietic PGD synthase inhibitor. There was reduction in cutaneous inflammatory responses, which was associated with decreased infiltration of eosinophils, basophils, lymphocytes, and RANTES and decreased production of macrophage-derived chemokines. By repeated hapten application in long-term contact hypersensitivity, CRTH2 deficiency resulted in decreased serum IgE production and approximately 50% of the skin responses. However, in vivo migration of dendritic cells and Langerhans cells to regional lymph nodes was not impaired in CRTH2-deficient mice. In contrast, the delayed-type hypersensitivity to sheep red blood cells and irritation dermatitis in mutant mice were the same as in wild-type mice.

Expression of CRTH2 in Mast Cells

Mast cells are present in all organs of the body and increase in sites of inflammation. Mast cells arise from CD34⁺/CD117⁺ pluripotent hematopoietic stem cells in the bone marrow and on growth factor stimulation mature to committed progenitors. Mast cells bind IgE via FcεRI, which is a high-affinity IgE receptor, and it is via FcεRI that mast cells are activated by allergens in allergic disease (Fig 3). Because mast cells are the largest sources of PGD₂ in the body, Boehme et al⁴⁸ hypothesized the expression of CRTH2 by mast cells. Mast cells synthesize and release PGD₂ on activation of the CRTH2–PGD₂ system that result in an autocrine feedback loop recruiting a greater number of mast cells to the sites of inflammation. They showed that primary murine bone marrow–derived mast cells express PGD₂ receptor CRTH2, which on activation by the CRTH2-specific agonist DK-PGD₂ results in calcium mobilization and phosphorylation of the p42/44 extracellular signal-regulated kinases and upregulation of CD23 and CD30 on the cell surface, as well as CD62L shedding. Thus, intradermal injection of DK-PGD₂ resulted in PGD₂, and DK-PGD₂ induced migration of bone marrow–derived mast cells both in vitro and in vivo. The CRTH2 antagonist inhibits both these processes. Phosphorylation of extracellular signal-regulated kinases could also be blocked by CRTH2 antagonist and pertussis toxin. These results show that PGD₂–CRTH2 interaction on mast cells plays an important role in allergic inflammation.

Role of CRTH2 in Allergic Inflammation

CRTH2 is selectively expressed in T_H2 cells but not in T_H1 cells among circulating CD4⁺ lymphocytes. A study by Iwasaki et al⁴⁹ investigated the correlation between CRTH2 and T_H2 cells among circulating T_H cells in healthy adults and patients with atopic dermatitis. Peripheral blood samples were taken from 40 patients with atopic dermatitis and 33 volunteers. The blood samples

revealed that CRTH2CD4 lymphocytes had a pure T_H2 phenotype. The peripheral blood CRTH2 CD4 lymphocytes were attracted by PGD₂ and the T_H2 -attracting chemokine thymus and the activation regulated chemokine (CCR4 agonist), whereas they showed little chemotactic or no significant migration toward the T_H1 -attracting chemokine interferon γ (IFN- γ) inducible protein 10, a CXCR3 agonist. The latter was noted only at higher concentrations and macrophage inflammatory protein 1 β (CCR5 agonist) at concentrations of 100nM. Thus, CRTH2⁺ T_H cells of healthy individuals expressed high levels of CCR4 but low levels of CXCR3 and CCR5. The number of CRTH2⁺ cells in the skin homing receptor cutaneous lymphocyte-associated antigen (CLA)-positive T_H cell compartment correlates with the severity of atopic dermatitis. The frequency of CLA⁺CRTH2⁺ cells was significantly increased, whereas there was no increase in CLA⁺CRTH2⁺ cells among CD4 lymphocytes in patients with atopic dermatitis compared with healthy individuals. Thus, the results suggest the involvement of the PGD₂/CRTH2 system in both normal and pathogenic T_H2 responses.⁴⁹

Mutalithas et al¹⁷ studied the expression of CRTH2 on T cells in blood and BAL fluid in healthy individuals and asthmatic patients.¹⁷ The study included 20 healthy individuals and 24 patients with asthma. The investigators showed that in blood only a small number of CRTH2⁺ T cells were seen in patients with asthma (1.8 ± 0.2) and healthy individuals (1.6 ± 0.2), and the percentage of CRTH2⁺ T cells was similar in asthmatic patients and healthy individuals. In blood, CRTH2 is augmented on IL-4- and IL-13-expressing T cells compared with IFN- γ T cells. There were a small number of CRTH2⁺ T cells in the BAL of both asthmatic patients and healthy individuals, but there were more CRTH2⁺ T cells in the BAL of asthmatic patients ($2.3\% \pm 0.6\%$) compared with healthy individuals ($0.3\% \pm 0.1\%$). In BAL from asthmatic patients, the expression of CRTH2 was enriched on IL-4 and IL-13 T cells compared with IFN- γ T cells. However, no difference was found in concentration of PGD₂ in the BAL between healthy individuals and asthmatic patients. Therefore, their data do not support a strong effect for CRTH2 in recruiting T cells to the lung.

Barnes et al⁸ performed a randomized, double-blind, placebo-controlled, parallel group study trial of the CRTH2 antagonist OCOO0459 in an outpatient population of patients with moderate persistent asthma. Patients were randomized, 65 patients were allocated and received OCOO0459, and 67 received the placebo. The objective of the study was to investigate the clinical effects of oral daily doses of 200 mg twice daily of OCOO0459 for 28 days compared with placebo. Improvement in the forced expiratory volume in 1 second was selected as the primary end point. In addition to assessing the safety and tolerability, the secondary objectives were to investigate the induced sputum differential eosinophil count, serum IgE levels, diary recorded peak expiratory flow, asthma symptoms, quality of life, salbutamol metered-dose inhaler use, and other clinic spirometry variables.

Results were analyzed for both a full analysis population and a per protocol population in which 55 were treated with OCOO0459 and 52 with placebo. The mean change in forced expiratory volume in 1 second in the full analysis population was 7.1% with OCOO0459 compared with 4.3% with placebo, which was not significant, whereas in the per protocol population the difference was statistically significant ($P = .037$). Greater improvement was seen in the daytime asthma symptoms, but this was not statistically significant; however, the greater improvement in nighttime symptoms was statistically significant. Improvement in the quality of life was seen in both the full analysis and per protocol population. However, the geometric mean sputum eosinophil count reduced significantly with OCOO0459, but when compared with placebo this finding did not reach statistical significance. A greater reduction was also seen in IgE after treatment with OCOO0459 compared with that observed with placebo. Adverse events were the same in both the OCOO0459 and placebo groups, but the respiratory infections were found to be less in OCOO0459-treated patients than in patients receiving placebo. To our knowledge, this is the first clinical study that provides evidence of the CRTH2 antagonist OCOO0459 as a novel oral treatment for asthma and related diseases (Table 2).⁸

Table 2
Clinical trials on CRTH2

Clinical trials.gov Identifier	Drug	No. of study participants	Patient age, y/ baseline characteristics	Type of study	Study duration, wk	Primary and secondary outcome measures	Result
NCT01057927	OC000459	65 in the OC000459 group and 67 in the placebo group	18–55/moderate persistent asthma	Multicenter, randomized, double-blind, placebo-controlled, parallel group study	12	Improvement in FEV ₁ is the primary end point, diary recorded peak expiratory flow, asthma symptoms, quality of life, salbutamol metered dose inhaler use, induced sputum differential eosinophil count, and serum IgE levels	Improvement in quality of life and nighttime symptom score and mean sputum eosinophil count; adverse events comparable to placebo
NCT00784732	QAV680	44; some were given QAV680, whereas others were given mometasone furoate	18–65/seasonal allergic rhinitis in environmental exposure chamber	Randomized, partially blinded, 2-way crossover, placebo-controlled, phase 2 study	16	Total nasal symptom score, measure of nasal cytokines (ECP, IL-5, IL-13, PGD ₂ , eotaxin), absolute eosinophil count from nasal lavage, nasal airway patency assessed by acoustic rhinometry, total ocular symptom score, assess the pharmacokinetics of plasma QAV680	Not available
NCT01448902	OC000459	36; some were given OC000459 and some were given placebo	18–50/grass pollen-induced allergic rhinitis	Randomized, double-blind, placebo-controlled, 2-way crossover, phase 2 study	12	Total nasal symptom score, assess safety via adverse events, clinical chemistry, hematology, vital signs, and FEV ₁ , assess plasma levels of OC000459	Not available

Abbreviations: ECP, eosinophil cationic protein; FEV₁, forced expiratory volume in 1 second; IL, interleukin; PGD₂, prostaglandin D₂.

Shirasaki et al.⁹ suggested that CRTH2 has an important role in recruitment of leukocytes into the allergic nasal mucosa. In 14 patients with nasal obstruction who were resistant to medical therapy, inferior turbinates were obtained after turbinectomy. They evaluated the number of CRTH2-expressing cells in allergic and nonallergic human nasal mucosa using an immunohistochemical technique done by means of anti-CRTH2 antibody and monoclonal antileukocyte antibodies. The immunohistochemical analysis revealed that CRTH2-expressing cells were eosinophils, mast cells, macrophages, T lymphocytes, epithelial cells, vascular endothelial cells, and submucosal glands in the nasal mucosa. In allergic nasal mucosa, the CRTH2 expression of these leukocytes is considerably upregulated compared with those in nonallergic nasal mucosa.

Perez-Novo et al.⁵⁰ suggested that PGD₂ produced by mast cells in immunologically activated nasal polyp tissue through a CRTH2-dependent mechanism promotes the migration of T_H2 cells. They obtained tissue explants from 10 patients with nasal polyposis, which were incubated with RPMI alone or RPMI containing IgE/anti-IgE. After stimulation with IgE/anti-IgE the transcript levels of DP1, CRTH2 receptors, and PGD₂ synthase increased. They reported considerably increased chemotaxis of T_H2 cells caused by supernatants from IgE/anti-IgE-stimulated nasal polyp tissue. The degree of T_H2 cell chemotaxis and the levels of PGD₂ produced were highly correlated. The production of T_H2 cell chemotactic activity was inhibited by diclofenac, and the chemotactic effect of the supernatant on T_H2 cells was inhibited by ramatroban. This study was the first to demonstrate the role of CRTH2 in nasal polyps, thus suggesting that CRTH2 antagonist may be useful in treating nasal polyps and other upper airway diseases.

Conclusion

PGD₂-CRTH2 interactions on mast cells play an important role in allergic inflammation. A recent clinical study provides evidence that the CRTH2 antagonist OCO00459 is a novel oral treatment for asthma and related diseases. CRTH2 mediates the activation of human T_H2 cells in response to PGD₂ released from IgE/anti-IgE-treated nasal polyp tissue. Thus, future studies of these receptors will lead to a new approach for treatment of allergic diseases.

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